CLAIMS

We Claim:

1. A method for generating a dopaminergic cell line stably expressing human A53T α-synuclein, the method comprising the steps of:

(a) introducing an expression vector into a population of rat pheochromocytoma PC12 cells, wherein the expression vector comprises a sequence encoding human A53T α-synuclein operatively linked to and under the control of a promoter; and

(b) isolating PC12 cell lines stably expressing human A53T α-synuclein.

2. The method of Claim 1, wherein the expression vector further comprises a sequence encoding a selectable marker gene.

3. The method of Claim 2, wherein the selectable marker gene is one of *neo*, *hyg*, *pac*, *zeo* or *gpt*.

4. The method of Claim 3, wherein the selectable marker gene is *neo*.

5. The method of Claim 2, wherein the PC12 cells stably expressing human A53T α-synuclein are isolated by virtue of growth on a selective medium, wherein growth on the selective medium indicates successful introduction and expression of the expression vector.

6. The method of Claim 5, wherein the selective marker gene is *neo* and the selective medium comprises neomycin.

7. The method of Claim 2, wherein the promoter is an inducible promoter.

8. The method of Claim 7, wherein the inducible promoter is selected from the group consisting of a tetracycline-responsive promoter, an ecdysone-inducible promoter, a metallothionein-regulated promoter, a steroid-regulated promoter, and a heat-shock regulated promoter.

9. The method of Claim 1, wherein the expression vector is a plasmid vector, a phasmid vector, an adenoviral vector, an adenoassociated vector, a vaccinia viral vector, a lentiviral vector, a herpes viral vector or a retroviral vector.

10. The method of Claim 9, wherein the expression vector is a plasmid vector.

11. The method of Claim 10, wherein the plasmid vector is pcDNA3.

12. The method of Claim 2, wherein the expression vector is introduced *via* calcium phosphate transfection, liposome-mediated transfection, microprojectile-mediated delivery, electroporation, biolistic transfection, microinjection, or viral-mediated transfection.

13. The method of Claim 12, wherein the expression vector is introduced *via* electroporation.

14. The method of Claim 1, wherein a cell of the isolated cell line exhibits proteasomal dysfunction as compared to a cell from a suitable control cell line not expressing mutant α-synuclein.

15. The method of Claim 14, wherein proteasomal dysfunction is determined by the increased presence of ubiquinated aggregates in the cytoplasm of a cell of the isolated cell line, as compared to a cell from a suitable control cell line not expressing mutant α-synuclein.

16. The method of Claim 14, wherein proteasomal dysfunction is determined by a significantly decreased proteasomal chymotrypsin-like activity in a cell of the cell line, as compared to a cell from a suitable control cell line not expressing mutant α-synuclein.

17. The method of Claim 1, wherein a cell of the isolated cell line exhibits dopaminergic dysfunction, as compared to a cell from a suitable control cell line not expressing mutant α-synuclein.

18. The method of Claim 17, wherein dopaminergic dysfunction is determined by an absence of dense core granules in a cell of the cell line.

19. The method of Claim 17, wherein dopaminergic dysfunction is determined by an absence of evoked dopamine release by a cell of the cell line.

20. The method of Claim 1, wherein a cell of the isolated cell line exhibits lysosomal dysfunction, as compared to a cell from a suitable control cell line not expressing mutant α-synuclein.

21. The method of Claim 20, wherein lysosomal dysfunction is determined by the accumulation of lysosomal-autophagic structures in a cell of the cell line.

22. The method of Claim 1, wherein cells of the isolated cell line exhibit increased non-apoptotic cell death, as compared to a cell from a suitable control cell line not expressing mutant α-synuclein.

23. The method of Claim 1, wherein a cell of the isolated cell line exhibits (i) proteasomal dysfunction, (ii) dopaminergic dysfunction, (iii) lysosomal dysfunction and (iv) increased non-apoptotic cell death, as compared to a cell from a suitable control cell line not expressing mutant α-synuclein.

24. A PC12 cell line stably expressing human A53T α-synuclein, wherein a cell of the cell line is characterized by: (i) proteasomal dysfunction; (ii) dopaminergic dysfunction; (iii) lysosomal dysfunction; and (iv) increased non-apoptotic cell death, as compared to a cell from a suitable control cell line not expressing mutant α-synuclein.

25. The PC12 cell line of Claim 23, wherein a cell of the cell line is further characterized by: (i) the presence of ubiquinated aggregates in the cytoplasm; (ii) significantly decreased proteasomal chymotrypsin-like activity; (iii) an absence of dense core granules; (iv) an absence of evoked dopamine release; and (v) an accumulation of lysosomal-autophagic structures.

26. A method for producing a cellular model of Parkinson's disease comprising the steps of:

(a) introducing an expression vector into a population of rat pheochromocytoma PC12 cells, wherein the expression vector comprises a sequence encoding human A53T α-synuclein operatively linked to and under the control of a promoter; and

(b) isolating PC12 cell lines stably expressing human A53T α-synuclein, wherein the cells of the isolated PC12 cell lines are characterized by (i) proteasomal dysfunction; (ii) dopaminergic dysfunction; (iii) lysosomal dysfunction; and (iv) increased non-apoptotic cell death.

27. The method of Claim 26, wherein the cells of the isolated PC12 cell lines are further characterized by: (i) the presence of ubiquinated aggregates in the cytoplasm; (ii) significantly decreased proteasomal chymotrypsin-like activity; (iii) an absence of dense core granules; (iv) an absence of evoked dopamine release; and (v) an accumulation of lysosomal-autophagic structures.

28. A method of identifying an agent that inhibits cellular degeneration associated with the expression of mutant α-synuclein, the method comprising the steps of:

(a) obtaining a PC12 cell line stably expressing human A53T α-synuclein, wherein cells of the cell line are characterized by increased non-apoptotic cell death;

(b) contacting the cells of the PC12 cell line with an agent of interest;

(c) comparing the phenotype of the contacted cells with the phenotype of cells from a suitable control line; and

determining the effect of the agent of interest on the contacted cells, wherein a phenotype of the contacted cells associated with reduced cellular degeneration indicates that the agent of interest has an inhibitory effect on cellular degeneration associated with the expression of mutant α-synuclein.

29. The method of Claim 28, wherein a reduction of non-apoptotic cell death in the contacted cells as compared to cells of a suitable control line indicates that the agent has

an inhibitory effect on cellular degeneration associated with the expression of mutant α-synuclein.

30. The method of Claim 28, where the cells of the cell line stably expressing human A53T α-synuclein are further characterized by: (i) proteasomal dysfunction; (ii) dopaminergic dysfunction; and (iii) lysosomal dysfunction.

31. The agent identified by the method of Claim 30.

32. A method of identifying an agent that inhibits dopaminergic dysfunction associated with the expression of mutant α-synuclein, the method comprising the steps of:

(a) obtaining a PC12 cell line stably expressing human A53T α-synuclein, wherein cells of the cell line are characterized by dopaminergic dysfunction;

(b) contacting the cells of the PC12 cell line with an agent of interest;

(c) comparing the phenotype of the contacted cells with the phenotype of cells from a suitable control line; and

(d) determining the effect of the agent of interest on the contacted cells, wherein a phenotype of the contacted cells associated with increased dopaminergic function indicates that the agent of interest has an inhibitory effect on dopaminergic dysfunction associated with the expression of mutant α-synuclein.

33. The method of Claim 32, wherein an increase of intracellular dopamine levels in the contacted cells as compared to cells of a suitable control line indicates that the agent of interest has an inhibitory effect on dopaminergic dysfunction associated with the expression of mutant α-synuclein.

34. The agent identified by the method of Claim 33.

35. The method of Claim 32, wherein the ability of a contacted cell to undergo evoked dopamine release indicates that the agent of interest has an inhibitory effect on dopaminergic dysfunction associated with the expression of mutant α-synuclein.

36. The agent identified by the method of Claim 35.

37. The method of Claim 32, wherein the presence of dense core granules in the cytoplasm of the contacted cells indicates that the agent of interest has an inhibitory effect on dopaminergic dysfunction associated with the expression of mutant α-synuclein.

38. The agent identified by the method of Claim 37.

39. The method of Claim 32, wherein the cells of the cell line stably expressing human A53T α-synuclein are further characterized by: (i) proteasomal dysfunction; (ii) lysosomal dysfunction, and (iii) increased non-apoptotic cell death.

40. A method of identifying an agent that inhibits proteasomal dysfunction associated with the expression of mutant α-synuclein, the method comprising the steps of:

(a) obtaining a PC12 cell line stably expressing human A53T α-synuclein, wherein cells of the cell line are characterized by proteasomal dysfunction;

(b) contacting the cells of the PC12 cell line with an agent of interest;

(c) comparing the phenotype of the contacted cells with the phenotype of cells from a suitable control line; and

(d) determining the effect of the agent of interest on the contacted cells,

wherein a phenotype of the contacted cells associated with increased proteasomal activity indicates that the agent of interest has an inhibitory effect on proteasomal dysfunction associated with the expression of mutant α-synuclein.

41. The method of Claim 40, wherein an increased proteasomal chymotrypsin-like activity in the contacted cells as compared to the cells of a suitable control line indicate that the agent has an inhibitory effect on proteasomal dysfunction associated with the expression of mutant α-synuclein.

42. The agent identified by the method of Claim 39.

43. The method of Claim 40, wherein a decrease in the presence of ubiquinated aggregates in the contacted cells as compared to the cells of a suitable control line indicate

that the agent has an inhibitory effect on proteasomal dysfunction associated with the expression of mutant α-synuclein.

44. The agent identified by the method of Claim 43.

45. The method of Claim 40, wherein the cells of the cell line stably expressing human A53T α-synuclein are further characterized by: (i) dopaminergic dysfunction; (ii) lysosomal dysfunction; and (iii) increased non-apoptotic cell death.

46. A method of identifying an agent that inhibits lysosomal dysfunction associated with the expression of mutant alpha-synuclein, the method comprising the steps of:

(a) obtaining a PC12 cell line stably expressing human A53T α-synuclein, wherein cells of the cell line are characterized by lysosomal dysfunction;

(b) contacting the cells of the PC12 cell line with an agent of interest;

(c) comparing the phenotype of the contacted cells with the phenotype of cells from a suitable control line; and

(d) determining the effect of the agent of interest on the contacted cells,

wherein a phenotype of the contacted cells associated with increased lysosomal activity indicates that the agent of interest has an inhibitory effect on lysosomal dysfunction associated with the expression of mutant α-synuclein.

47. The method of Claim 46, wherein a decrease of the presence of lysosomal-autophagic structures in the cytoplasm of the contacted cells as compared to the cells of a suitable control line indicates that the agent has an inhibitory effect on lysosomal dysfunction associated with the expression of mutant α-synuclein.

48. The agent identified by the method of Claim 47.

49. The method of Claim 46, wherein an increase of fine, punctate staining with an ionic dye in the contacted cells as compared to the cells of a suitable control line indicate

that the agent has an inhibitory effect on lysosomal dysfunction associated with the expression of mutant α-synuclein.

50. The agent identified by the method of Claim 49.

51. The method of Claim 46, wherein an increase of acidification and degradation of a lysosomal substrate in the contacted cells as compared to the cells of a suitable control line indicate that the agent has an inhibitory effect on lysosomal dysfunction associated with the expression of mutant α-synuclein.

52. The agent identified by the method of Claim 51.

53. The method of Claim 46, wherein the cells of the cell line stably expressing human A53T α-synuclein are further characterized by: (i) proteasomal dysfunction; (ii) dopaminergic dysfunction; and (iii) increased non-apoptotic cell death.

54. A method of screening an agent to determine its potential effectiveness in the treatment of a synucleinopathic neurodegenerative disorder, the method comprising the steps of:

(a) obtaining a PC12 cell line stably expressing human A53T α-synuclein, wherein cells of the cell line are characterized by: (i) proteasomal dysfunction; (ii) dopaminergic dysfunction; (iii) lysosomal dysfunction; and (iv) increased non-apoptotic cell death;

(b) contacting the cells of the PC12 cell line with an agent of interest;

(c) comparing the phenotype of the contacted cells with the phenotype of cells from a suitable control line; and

(d) determining the effect of the agent of interest on the contacted cells,

wherein a phenotype of the contacted cells associated with (i) decreased proteasomal dysfunction; (ii) decreased dopaminergic dysfunction; (iii) decreased lysosomal dysfunction; or (iv) decreased non-apoptotic cell death, when compared to the cells of a suitable control line, indicates that the agent of interest is potentially effective in treating a synucleinopathic neurodegenerative disorder.

55. The method of Claim 54, wherein the synucleinopathic neurodegenerative disorder is one of Parkinson's Disease, Dementia with Lewy Bodies, Lewy Body Variant of Alzheimer's Disease, Multiple System Atrophy and Hallervorden-Spatz syndrome.

56. The method of Claim 55, wherein the Multiple System Atrophy is one of Shy-Drager Syndrome, striatonigral degeneration, and olivopontocerebellar atrophy.

57. The method of Claim 55, wherein the disease is Parkinson's Disease.

58. The agent identified by the method of Claim 57.